Minireview

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Natural History and Inherited Disorders of a Lysosomal Enzyme, β-Hexosaminidase*

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β-Hexosaminidase is one of the 40 or more enzymes that reside in lysosomes and participate in the degradation of glycoproteins, glycolipids, and glycosaminoglycans. Its role is to hydrolyze terminal β-linked GlcNAc and GalNAc residues. As for other lysosomal enzymes (1), the relative simplicity of the catalytic reaction contrasts with an elaborate system for delivery of active β-hexosaminidase to lysosomes. Mutations that lead to absence or malfunction of the enzyme result in lysosomal storage of its substrates, with consequences that are particularly damaging to the nervous system. This short review will focus on the cell biology of normal human β-hexosaminidase and on the molecular genetics of β-hexosaminidase deficiency diseases. The reader is referred to Sandhoff *et al.* (2) for a comprehensive review of the field.

Natural History

Genetics—The two subunits of human β-hexosaminidase, α and β, are encoded by separate genes located on chromosomes 15 and 5, respectively. The genes are 35–40 kilobases long and have similar architecture; 12 of the 13 introns interrupt the coding regions at analogous positions (3, 4). The coding regions, about 1600 bases long, show nearly 60% identity of nucleotide and deduced amino acid sequences (4–6). Because of their structural similarity, the two genes are thought to have evolved from a common ancestral gene. In keeping with this hypothesis, the amino acid sequence of the mouse β-subunit has 51 and 72% identity with the sequences of the human α- and β-subunits, respectively (7), and the sequence of the single polypeptide of *Dictyostelium discoideum* β-hexosaminidase has over 30% identity with the sequences of the two human subunits (8). Those parts of the sequences that have been particularly well conserved may be essential for folding and catalytic activity.

Biosynthesis—As is characteristic of proteins destined for lysosomes, the two subunits of β-hexosaminidase are transported through the endoplasmic reticulum and Golgi. They undergo numerous post-translational modifications in transit or after reaching lysosomes, including removal of the signal peptide, *N*-glycosylation, formation of disulfide bonds, acquisition of the mannose 6-phosphate recognition marker, and limited proteolysis (1). The structures of the mature subunits therefore differ significantly from those of the newly synthesized polypeptides. Some of the major changes (proteolysis and glycosylation) are summarized in Fig. 1.

The amino acid sequences deduced from the α- and β-subunit cDNAs predict signal peptides 22 and 42 amino acids long, as well as three and five potential *N*-glycosylation sites, respectively. The correct assignment of the signal peptide cleavage sites has been verified by Edman degradation of the α- and β-polypeptides made in cell-free translation in the presence of microsomes (9, 10) and of the precursor forms isolated from intact fibroblasts or fibroblast secretions (9–11). Expression in Cos-1 cells of α- and β-cDNAs mutagenized at each potential glycosylation site showed that all but one of the sites were used (12).[1] The mutagenesis experiments (12) also showed that the first and fourth glycosylation sites of the β-subunit preferentially acquired the mannose 6-phosphate marker for targeting to lysosomes; the phosphorylated oligosaccharide sites of the α-subunit have not yet been mapped.

The earliest evidence for proteolytic modification beyond removal of the signal peptide came from metabolic labeling studies in cultured fibroblasts, which identified larger precursor and smaller mature forms of both subunits (13). The sites of proteolytic cleavage were subsequently identified by Edman degradation of α- and β-polypeptides that had been biosynthetically radiolabeled in cultured fibroblasts for varying periods of time (9, 10) or that had been derived from enzyme purified from tissues (14). The processing of the two subunits is different; the α-subunit loses 67 amino acids from the amino end, whereas the β-subunit precursor undergoes internal cleavages but the three pieces remain attached by disulfide bonds (Fig. 1). The larger fragments, *a* and *b*, correspond to β_a and β_b, respectively, that have been isolated from purified placental enzyme (14); the smaller *c* fragment has been reported to date only in enzyme radiolabeled in fibroblasts. These proteolytic modifications represent changes which occur in the degradative environment of lysosomes, but they are not required for catalytic activity; for example, β-hexosaminidase found in fibroblast secretions is fully active even though both subunits are in precursor form (15).

Oligosaccharide chains are also extensively degraded in lysosomes. Structural analysis of oligosaccharides from placental β-hexosaminidase showed that some of the *N*-linked oligosaccharides had 5–7 mannose residues, whereas others had only 3 (16). The oligosaccharide at position 327 of the β-subunit, shown in transfection studies (12) to be phosphorylated (and by implication, to have a high mannose structure), was present in tissue enzyme as an *N*-acetylglucosamine stub (16). There is evidence that the α-subunit is sialylated during biosynthesis and that the sialic acid is subsequently removed (9). Sulfate is also transiently present on complex oligosaccharides of the α-subunit (17).

A post-translational event of particular importance to β-hexosaminidase is the dimerization of its subunits to give catalytically active enzyme. Neither the α-monomer (18) nor the β-monomer (19) is catalytically active. Perhaps because they are structurally similar, the monomers can associate in three ways to give homo- or heterodimeric isoenzymes: A ($\alpha\beta$), B ($\beta\beta$), and S ($\alpha\alpha$). The A isoenzyme has the broadest substrate specificity and is absolutely essential for neuronal function, as will be discussed in the section on inherited disorders.

The α- and β-subunits are synthesized in cultured cells in approximately equal amounts, as judged by early incorporation of radiolabel (13, 18), but they dimerize at different rates.

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[1] G. Weitz and R. Proia, personal communication.

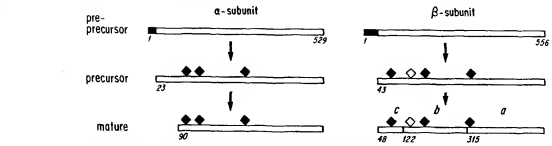

FIG. 1. Limited proteolysis and glycosylation of the two subunits of β-hexosaminidase. The α- and β-polypeptides are represented by *bars*, with the terminal amino acids designated by *numbers*. The *a*, *b*, and *c* fragments of the mature β-subunit are joined by disulfide bonds. ■, signal peptide; ◆, *N*-glycosylation site; ◇, site of incomplete *N*-glycosylation. Data adapted from Refs. 10, 12, and G. Weitz and R. Proia, personal communication.

Pulse-chase experiments have shown that the pool of radioactive β-monomers is depleted within an hour (19), whereas that of α-monomers takes 6–10 times longer (18). The rate-limiting step in the association of α-subunits is not known; it may be folding, disulfide bonding, or some yet unidentified post-translational modification. The site of ββ dimer formation is thought to be the endoplasmic reticulum, in part because of the rapid rate at which it occurs in intact cells and in part because it occurs in a cell-free translation system in the presence of microsomes (19). On the other hand, the site of αβ association is not clear. It had been attributed to the Golgi because it occurred after incorporation of ^{32}P to form the mannose 6-phosphate recognition marker (18). At the time, phospho-*N*-acetylglucosamine transferase, the enzyme responsible for this incorporation, had been localized to the *cis*-Golgi (1). However, some phospho-*N*-acetylglucosamine transfer has since been found to occur in the endoplasmic reticulum (20). Thus the available data do not allow unambiguous assignment of αβ dimerization to the Golgi nor to the endoplasmic reticulum. Though oligomerization is required for exit from the endoplasmic reticulum in other systems (21, 22), this may not be the case for the α-subunit, which can be found in monomeric form in fibroblast secretions (18).

Inherited Disorders

Pathogenesis—The β-hexosaminidase deficiency diseases can be readily understood in the context of the different substrate specificities of the three isoenzymes. The A ($\alpha\beta$) isoenzyme has the broadest specificity; it can remove nonreducing terminal GlcNAc and GalNAc residues from all glycopeptides, glycosaminoglycans, and glycolipids that occur in human cells. The B ($\beta\beta$) isoenzyme has similar substrate specificity with the key exception that it does not hydrolyze G_{M2} ganglioside.[2] The S ($\alpha\alpha$) isoenzyme has generally limited catalytic activity and is unstable. If the A isoenzyme activity is lost because of mutation in either the α- or β-subunit gene, it cannot be replaced by the action of either the B or S isoenzymes, and G_{M2} ganglioside accumulates in lysosomes. This is particularly damaging to the nervous system, where the glycolipid is an important plasma membrane constituent that must be continuously turned over. In cases of α-subunit mutations that result in complete β-hexosaminidase A deficiency, massive lysosomal accumulation of G_{M2} ganglioside causes neurons to balloon or to develop special structures (meganeurites) to accommodate the stored material. The resulting neuronal malfunction and degeneration leads to seizures, blindness, loss of all intellectual and cognitive abilities, and early death. Mutations in the β-subunit gene cause loss of both the A and B isoenzymes and therefore accumulation of water-soluble *N*-acetylglucosaminides and *N*-acetylgalactosaminides in addition to the G_{M2} ganglioside. As the consequences of G_{M2} ganglioside accumulation in the brain overshadow those of storage of water-soluble materials in other tissues, the clinical course of A and B isoenzyme deficiency (Sandhoff disease) is similar to the course of A isoenzyme deficiency alone (Tay-Sachs disease). Yet another biochemically distinct but clinically similar disease results from mutation of the gene encoding a lipid binding protein (called the "G_{M2} activator protein") that complexes G_{M2} ganglioside stoichiometrically for hydrolysis by β-hexosaminidase A.

Whereas mutations that cause complete loss of β-hexosaminidase A activity give rise to devastating disease, mutations that leave some residual activity give rise to disease of later onset and milder course and on occasion to an asymptomatic state. The severity of the disease can be inversely correlated with the level of residual activity, provided the latter is measured under conditions that approximate the *in vivo* hydrolysis of G_{M2} ganglioside (23).

In spite of its negligible catalytic activity toward physiologic substrates, the S isoenzyme shares with the A the ability to remove *N*-acetylglucosamine 6-sulfate *en bloc* (24). Kinetic experiments suggest that this reaction, which is unusual for mammalian glycosidases, is a property of the α-subunit site that hydrolyzes G_{M2} ganglioside (25). On the other hand, the β-subunit appears to have a catalytic site only for neutral *N*-acetylhexosaminides. By this hypothesis, the α- and β-subunits have distinct catalytic sites but act in concert, as neither is active in monomeric form. These interesting enzymologic findings have provided more specific reagents for diagnosis and better understanding of the disorders.

The biochemistry and pathogenesis of the various β-hexosaminidase deficiency diseases are reviewed in Ref. 2.

α-Subunit Mutations (β-Hexosaminidase A Deficiency)—Infantile Tay-Sachs disease is the consequence of a complete lack of β-hexosaminidase A activity. Historically, the disease was thought to occur almost exclusively in the Ashkenazi Jewish population. About 3% of Ashkenazi Jews in the United States are carriers for β-hexosaminidase A deficiency. As the voluntary carrier detection programs of the last 20 years have all but eliminated the disease in that group, attention has been called to a significant number of cases in the general population. Molecular characterization of the mutations has shown that they can occur over the entire gene (Fig. 2) and produce absent or defective α-subunit by many mechanisms.

The major α-subunit mutation in the Ashkenazi population is an insertion of 4 bases into exon 11 (26). The reading frame

[2] The abbreviation used is: G_{M2} ganglioside, *N*-acetylgalactosaminyl-β1→4-(*N*-acetylneuraminyl-α2→3)-galactosyl-β1→4-glucosyl-β1→1-ceramide.

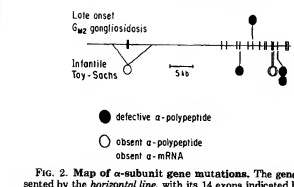

FIG. 2. **Map of α-subunit gene mutations.** The gene is represented by the *horizontal line*, with its 14 exons indicated by *vertical bars* (3). Mutations characterized as of April 1989 are represented by *solid* or *open circles*, depending on whether they cause the presence or absence of α-polypeptide and are placed below or above the gene depending on the clinical consequences. From *left* to *right*, the indicated mutations are: (*a*) deletion of exon 1 and flanking sequences, found in French-Canadians; (*b*) a one-nucleotide substitution in exon 5, found in patients with the B1 phenotype; (*c*) a one-nucleotide substitution in exon 7, found in late-onset G_{M2} gangliosidosis patients of Ashkenazi Jewish origin; (*d*) a four-nucleotide insertion in exon 11, the major mutation in the Ashkenazi Jewish population; (*e*) a splice site mutation in intron 12, a minor mutation in that population; (*f*) and (*g*) a one-nucleotide substitution in exon 13 and a one-nucleotide deletion with premature termination, also in exon 13.

is shifted and a termination codon occurs 9 nucleotides downstream. There is an almost complete absence of mature mRNA, although nuclear run-on transcription is normal (27).[3] It is not clear why a termination codon four-fifths of the way to the carboxyl terminus would result in a deficiency of mRNA rather than in a truncated polypeptide. The anomaly has precedents in the thalassemias where it has been attributed to defective transport or instability of the nuclear mRNA (28, 29). Alternatively, the allele with the 4-nucleotide insertion in exon 11 may carry an additional mutation that would be responsible for the absence of mRNA.

Another mutation found in the Ashkenazi population is a splice site mutation, a G → C transversion at the 5′ border of intron 12 (30–32). As expected, this change in a highly conserved sequence does not permit normal splicing, and the level of mature mRNA is very low. Abnormal species of mRNA, including some with exon 12 deleted, have been found (33).

A deletion of the first exon, together with flanking sequences, has been found in homozygosity in some patients of French-Canadian origin (34). The 7.6-kilobase deletion may have occurred by homologous recombination of misaligned Alu sequences (35). There may be additional α-subunit mutations in the French-Canadian population (36) among whom the frequency of heterozygotes is nearly as high as among Ashkenazi Jews.

Other mutations identified in the α-subunit gene are in the coding region and give rise to defective polypeptides. Two different mutations in exon 13 result in defective α-subunits that do not exit from the endoplasmic reticulum: a one-base change that results in substitution of glutamate 482 to lysine (37, 38) and a one-base deletion that causes premature termination and loss of 23 amino acids (39). These changes near the carboxyl terminus may cause misfolding, which in turn would cause retention in the endoplasmic reticulum, as has been found in other systems (40). As the mutations were found in Tay-Sachs patients whose parents were consanguinous (by coincidence, both families were of Italian origin) they are probably very rare, perhaps limited to the kindreds in which they were identified.

An interesting mutation called "B1" affects the catalytic properties of the A isoenzyme so that it can hydrolyze neutral substrates but not G_{M2} ganglioside or other acidic substrates. It has been identified as a missense mutation in exon 5 which changes arginine 178 to histidine (41). The substitution may affect the catalytic site of the α-subunit directly or indirectly, perhaps by altering the conformation of the polypeptide. The same mutation has been found in five of six B1 patients of different geographic and ethnic origin (42). Such repeated finding of a rare mutation in unrelated populations indicates that the biochemical abnormality that defines B1 can be produced in only a limited number of ways.

Recently, a mutation responsible for late-onset G_{M2} gangliosidosis has been identified as a G → A transition in exon 7, which causes the substitution of serine for glycine 269 (43, 44). The altered α-suhunit associates poorly with the β-subunit (45). A low level of residual activity allows the patients to escape the neurodegeneration characteristic of infantile Tay-Sachs disease but results in motor neuron disease occasionally accompanied by psychosis. The onset of the disease is usually in adolescence or adulthood, and patients may lead productive lives for many decades. There is marked variability of clinical manifestations, even within families, suggesting that other genetic factors or perhaps environmental ones can affect the residual enzyme activity. The patients studied to date have all been of Ashkenazi origin, and the exon 7 mutation was found in compound heterozygosity with the allelic mutations in exon 11 or intron 12 that are known to occur in the Ashkenazi population.

β-Subunit Mutations (β-Hexosaminidase A and B Deficiency; Sandhoff Disease)—These have not yet been studied in the same detail as the α-suhunit mutations. Predictably, they are heterogeneous; deletion and nondeletion, absence and presence of mRNA, have all been found (46). An insertion of several amino acids as a result of splicing part of intron 12 or 13 into the coding sequence has been found in two patients with juvenile Sandhoff disease and two asymptomatic individuals (47).[4] This abnormality near the carboxyl terminus causes retention of the polypeptide in the endoplasmic reticulum (48). As was found for the α-subunit, the carboxyl terminus of the β-suhunit appears to be essential for correct folding and transport.

Concluding Remarks—Questions concerning the origin and spread of mutations of the α-subunit gene can now be answered by comparing the types of mutations among enzymatically proven heterozygotes of Ashkenazi, French-Canadian, and other ethnic origins. Selection in favor of partial deficiency of β-hexosaminidase A isoenzyme has been proposed to account for the very high carrier rate in certain populations, hut except for the hypothesis of increased resistance to tuberculosis (49, 50) no selective mechanism has been proposed.

Because of the heterogeneity of mutations leading to deficiency of the A isoenzyme of β-hexosaminidase, DNA-based tests are not likely to supplant current diagnostic procedures which are based on measurement of enzyme activity. However, in appropriate situations, DNA-based assays can be used to differentiate between mutations that would cause infantile Tay-Sachs or Sandhoff disease from those that would result in less severe forms of G_{M2} gangliosidosis.

The recent molecular advances offer hope for eventual therapy of the G_{M2} gangliosidoses. Production of large quantities of enzyme in specially engineered cells, modification of the enzyme for targeting to neurons, and replacement or

[3] All the mutant cell strains used for nuclear run-on transcription (27) were subsequently shown to be homozygous for the exon 11 mutation (B. Paw and E. F. Neufeld, unpublished experiments).

[4] B. Dlott and E. F. Neufeld, unpublished results.

repair of the mutant gene are directions that surely will be pursued in the years ahead.

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